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Commercial fertilizer as effective iron chelate (Fe^{3+}-EDDHA) for wastewater disinfection under natural sunlight for reusing in irrigation

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ABSTRACT

In this study, the use of a commercial iron fertilizer (Fe^{3+}-EDDHA) employed to remediate iron chlorosis in agriculture has been investigated as a promoting bactericidal agent in solar wastewater disinfection processes. Two water matrices: isotonic water (IW) and synthetic fresh-cut wastewater (SFCWW) and two bacterial strains (*E. coli O157:H7* and *Salmonella enteritidis*) have been investigated. The bacterial inactivation rates were compared with other solar processes (solar only, H_2O_2/solar, Fe^{3+}/solar and Fe^{3+}/H_2O_2/solar) at neutral pH and at laboratory scale (200 mL) under natural solar radiation. Reagents concentration tested was 0.5, 2.5 and 5 mg L^{-1} of Fe^{3+} or Fe^{3+}-EDDHA and 1, 5 and 10 mg L^{-1} of H_2O_2.

Microbial inactivation kinetics showed an improvement of the solar disinfection efficiency when using Fe^{3+}-EDDHA/solar in comparison with Fe^{3+}/H_2O_2/solar (conventional photo-Fenton) in both water matrices. Among all reagent concentrations tested, the best inactivation kinetic rate for both bacteria was obtained with 2.5/5 mg L^{-1} Fe^{3+}-EDDHA/H_2O_2, reaching > 5-log reduction in 45 min of treatment or 31 Whm^{-2} of solar UVA-dose. In addition, an inactivation mechanism has been proposed based on changes in membrane permeability when Fe^{3+}-EDDHA is present and on structural damages caused by hydroxyl radicals ($HO^{\bullet}$) for Fe^{3+}-EDDHA/H_2O_2/solar process.

Finally, this study highlights the possibility of efficient fresh-cut wastewater treat for further irrigation reuse in arid and semiarid regions using disinfected wastewater that already includes iron fertilizer, reducing water scarcity and with the additional advantage of diminished impact of iron chlorosis in crops.

1. Introduction

The Mediterranean region is undergoing rapid global socio-economic changes that involve important environmental problems like the water scarcity [1]. Agriculture plays a critical role in the Mediterranean water imbalances with more than 50% of the fresh water resources consumption. The intensification of water stress generates new water-food challenges to current and future sustainability agriculture [1]. Therefore, the implementation of emerging technologies to respond to these pressures in water-scarce countries is crucial for water management. In this regard, industrial wastewater (WW) reuse in agriculture represents an unconventional water supply, improving the water use efficiency in Mediterranean countries. Besides, the implementation of a strategy to treat and reuse WW from agro-food industries in agriculture will allow a reduction of the water footprint of these types of industries.

Among the different agro-food industries, the fresh-cut produce industry stands out for its rapid development in the last years due to the trend to consume healthy, fresh and easy to prepare food [2]. The rapid market growth and therefore, the increase in consumption of fresh-cut products are associated with the increase of foodborne illness outbreaks linked to this industry in the last decades. Enterohemorrhagic *E. coli* O157:H7 and *Salmonella* spp. are two of the most frequent and important faecal pathogens associated with foodborne illness in fresh-cut industry [3]. This industry uses water in numerous steps of the process and therefore is one of the major water consumers of the agro-food sector (ca. 2–11 m^3ton^{-1} of product) [4].

Chlorine compounds are the most commonly used water disinfectant in this industry due to their low price, easy application and high antimicrobial effectiveness, with concentrations ranged between 50 and 200 mg L^{-1} of free chlorine [5]. Nevertheless, the use of chlorinated compounds in fresh-cut industry has been forbidden in some European countries mainly due to the generation of unhealthy and toxic

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compounds as a result of the chemical reaction between chlorine and the organic matter present in the washing water [5]. In addition, the residual free chlorine in this wastewater at higher concentrations than the established in guidelines ($< 1\ mg\ L^{-1}$) prevents its possible reuse for irrigation [6].

Consequently, the search and evaluation of alternative disinfection procedures has grown in importance in light of the recent European trend of banning the chlorination process in fresh-cut industry. In the last years, different water disinfection treatments have been proposed and evaluated to replace chlorination, including electrolyzed oxidizing water, weak organic acids, membrane filtration, ultrasound, UV-C light, etc. All these alternative treatments are capable of reducing the microbial load but present two important drawbacks: they are not completely efficient and some of them may modify the organoleptic properties of the fresh-cut product which is one of the most important features in this type of food industry [5]. A common alternative is the use of advanced oxidation processes (AOPs) such as ozone, ozone/UVC or UVC/TiO_2 [7,8]. However, the high process costs represent one of the main obstacles to their commercial application. Employing solar driven AOPs processes represents an environmentally friendly alternative, where solar photo-Fenton process has demonstrated a high efficiency for water purification [9]. Recently, the disinfection efficiency of solar photo-Fenton and H_2O_2/solar has been investigated as a potential alternative to the use of chlorine in fresh-cut industry, showing successful disinfection capability at neutral pH [4]. Nevertheless, it is very well known that the efficiency of photo-Fenton at neutral pH is strongly reduced due to iron precipitation. Research on new sources of iron as well as iron complexation with organic compounds to increase the capability of photo-Fenton at neutral pH has lately arisen [10]. New complexes based on iron chelates and even other metals like copper have been recently reported in literature for water and wastewater disinfection at neutral pH [10,11].

Several types of iron chelating agents have been reported in literature to enhance water disinfection and decontamination at near neutral pH by photo-Fenton trough keeping iron in solution, including aminopolicarboxylic acids like EDTA (Ethylenediaminetetraacetic acid) and EDDS (Ethylenediamine-N,N‘-disuccinic acid) [12]. On the other hand, the use of synthetic iron fertilizers based on aminopolycarboxylic acids are commonly used in Mediterranean agriculture as iron chelating agent to increase its bioavailability for plants and to avoid the well-known iron chlorosis, plant disease that reduces the crop yield [13]. Among the different iron chelating agents authorized by EC Regulation No. 2003/2003 and subsequent amendments, Ethylenediamine-*N,*N'-bis (2-hydroxyphenyl)acetic acid (EDDHA) is the most efficient to prevent and remedy iron chlorosis under neutral and alkaline soil conditions due to its high stability in a wide range of pH (3–10) [14]. Currently, 80% of fertilizers used in agriculture are synthetic iron chelates with 56–79% of EDDHA. In addition, the effect of the sub-products generated by photodecomposition has been previously investigated resulting non-toxic for crops [15]. Therefore, its use as possible iron-chelate for wastewater treatment and further reuse in agriculture seems to be a plausible option.

The aim of this work was to evaluate the efficiency of Fe^{3+}-EDDHA, for wastewater disinfection using a commercial fertilizer containing iron chelate. Proof-of-principle was investigated in isotonic water (IW) and synthetic fresh-cut wastewater (SFCWW) at neutral pH. The inactivation of *E. coli* O157:H7 and *Salmonella enteritidis* with Fe^{3+}-EDDHA/solar and in combination with H_2O_2 (Fe^{3+}-EDDHA/H_2O_2/solar) was evaluated. In addition, other solar processes including solar photo-inactivation, H_2O_2/solar, Fe^{3+}/solar and traditional photo-Fenton process (Fe^{3+}/H_2O_2/solar) using iron salts were simultaneously investigated. A comparative analysis of disinfection capability of all the solar processes was done in order to establish the suitability of the commercial fertilizer as alternative source of iron for agro-food wastewater disinfection and its possible reuse in agriculture.

2. Materials and methods

2.1. Water matrices

Two types of water were used: i) Isotonic water (IW): distilled water containing 0.9% NaCl w/v to avoid bacterial osmotic stress. This water matrix was used to investigate the efficiency of Fe^{3+}-EDDHA without any other chemical interactions in the process and ii) Synthetic fresh-cut wastewater (SFCWW) prepared according to [4] and characterized by $25.4 \pm 0.4\ mg\ L^{-1}$ of Dissolved Organic Carbon (DOC), 100.1 ± 0.4 NTU of turbidity, 6.25 ± 0.06 of pH and $1209.6 \pm 14.8\ \mu S\ cm^{-1}$ of conductivity.

This water matrix was used instead of real industrial WW to avoid influence of physic-chemical fluctuation of real WW sample, allowing a realistic comparative analysis between the treatments and operational conditions investigated.

2.2. Reagents

Two sources of Fe^{3+} were used: ferric nitrate salt ($Fe(NO)_3{\cdot}9H_2O$ (Panreac, Spain) and the commercial micronutrient Sequestrene 138 Fe G100 (Syngenta, Spain), both used as received from manufacturer. The data sheet of Sequestrene shows presence of a 7% of iron of which ca. 90% is chelated as Fe^{3+}-EDDHA. The Fe^{3+} concentrations tested for both iron sources (iron salt and Fe^{3+}-EDDHA) were 0.5, 2.5 and $5\ mg\ L^{-1}$. They were selected based on a previous work [4] and also considering the range of concentration of Sequestrene employed in intensive agriculture in the Southeast of Spain. Hydrogen peroxide (35% w/v, Merck, Germany) was used as received from the manufacturer and used at Fe:H_2O_2 concentration ratio of 1:2.

2.3. Analytical measurements

Iron and H_2O_2 concentration were measured using colorimetric methods: 1,10-phenanthroline as complexing agent to measure the iron concentration according to ISO 6332 and Titanium(IV)Oxysulfate (Riedel-de-Haën, Germany) to measure H_2O_2 concentration based in the formation of pertitanic acid [4]. A solution of bovine liver catalase at $0.1\ g\ L^{-1}$ (Sigma-Aldrich, USA) was added to the samples to eliminate residual H_2O_2 at 1:50 (catalase solution/sample).

Physicochemical characterization of water samples and Sequestrene solution was carried out using: turbidimeter (Model 2100 N, Hach, USA), pH meter (multi720, WTW, Germany), conductivity meter (GLP31, CRISON, Spain) and a TOC analyzer (Model 5050, Shimadzu, Japon). The content of ions, acids and amines was measured by ionic chromatography (Model 850, Metrohm, Switzerland).

Liquid chromatography was used for phenol detection using an Agilent 1260 (Palo Alto, USA) with a diode array detector (UV-DAD) and C-18 column (XDB-C18 Agilent 1.8 µm, 4.6 x 50 mm), flow rate of $1\ mL\ min^{-1}$ and 100 µL of injection volume. Elution method was in isocratic conditions with 20–80% of acetonitrile-acid ultrapure water (25 mM formic acid) during 5 min, phenol was detected at 268 nm and 1.9 min of retention time.

2.4. Bacterial enumeration

E. coli O157:H7 (CECT 4972) and *Salmonella* subsp. *Enteritidis* (CECT 4155) were obtained from the Spanish Culture Collection (CECT). The bacteria were cultured in Nutrient-Broth Agar I (containing $5\ g\ L^{-1}$ of NaCl (Sigma Aldrich) and $5\ g\ L^{-1}$ of beef extract and $10\ g\ L^{-1}$ of peptone (Panreac, Spain)) and Tryptone Soya Broth (OXOID) for *E. coli* and *S. enteritidis,* respectively. After incubation in a rotary shaking incubator at 37 °C and 100 rpm for 20 h, the suspensions obtained (concentration ca. $10^9\ CFUmL^{-1}$) were centrifuged for 10 min at 900 x*g*. Then, both bacterial pellets were re-suspended in phosphate-buffered saline (PBS) solution and directly diluted in the sample to obtain an

initial concentration of 10^6 CFUmL^{-1}/pathogen.

Water samples from solar experiments were serial diluted in PBS and enumerated using the standard plate counting method. 50 µL and 500 µL of samples were spread on ChromoCult® Coliform Agar (Merck KGaA, Darmstadt, Germany) and Salmonella Shigella Agar (Scharlau®, Spain) and incubated 24 h and 48 h at 37 °C for *E. coli* and *S. enteritidis,* respectively. Detection limit was 2 CFUmL^{-1}.

2.5. Sequestrene solution characterization

Photostability of Sequestrene solution was investigated using a solar simulator (Atlas Suntest XLS+, USA). Experiments were carried out in an open glass vessel reactor (19 cm diameter) magnetically stirred with an illuminated surface of 0.0284 m^2 and 700 mL of irradiated volume. 30 Wm^{-2} of UVA radiation intensity was selected since this irradiance is considered a mean value of global UV irradiance under clear skies in sunny countries [9].

The concentration of fertilizer selected for this analysis (100 mg L^{-1}) was done according to the data provided by the manufacturer as maximum concentration of total dissolved iron (≈7%). Iron analysis indicated a content of 7.5 mg L^{-1} of total iron, i.e. 6.2% in solution, which agrees with the commercial data provided by the manufacturer. A physic-chemical characterization of this solution was performed and data obtained were: Cl^- (17 mg L^{-1}); Na^+ (14 mg L^{-1}); SO_4^{2-} (0.4 mg L^{-1}); NO_3^-, K^+, glycolate, formate and trimethylamine (0.2 mg L^{-1}) and oxalate (0.1 mg L^{-1}). pH (7.3); conductivity (73 µS cm^{-1}); turbidity (5.7 NTU) and DOC (27.8 mg L^{-1}).

The generation of HO$^\bullet$ by Fe^{3+}-EDDHA/H_2O_2/solar has been investigated according to [16] in batch-vessel reactor with 200 mL ultrapure water spiked with benzene (2.95×10^{-3} M) and Fe^{3+}-EDDHA/H_2O_2 (2.5/5 mg L^{-1} of Fe^{3+} and H_2O_2), exposed to natural solar radiation for 3 h. The detection of phenol (indirect measurement of HO$^\bullet$ generation) was analyzed by liquid chromatography.

2.6. Solar disinfection experiments

Solar disinfection experiments were performed in 250 mL batch-vessel reactors (DURAN-Glass, Schott, Germany) in completely sunny days at Plataforma Solar of Almeria (latitude: 37.0909 °N, longitude: 2.357 °W). Reactors were covered by a glass cap (Schott) to allow the solar radiation enters from all directions and magnetically stirred at 450 rpm. Irradiated and total volume was 200 mL with 0.0095 m^2 of irradiated surface.

Reagents and microbial suspensions were directly and simultaneously diluted to obtain the desired initial concentrations. After 5 min of homogenization in the dark, the first sample (Time 0) was taken and the reactors were exposed to sunlight. Experiments started between 10:30 – 11:00 am local time lasting 4 h of solar exposure, water samples were taken at regular intervals for bacterial enumeration. Water temperature was monitored and ranged from 24.3 ± 1.9 °C to 38.6 ± 2 °C. Water pH was 6.9 ± 0.1 in all cases, remaining constant along the treatment time. DOC concentration was measured at the beginning and at the end of each solar experiment. In any case, the initial values of DOC in IW (only in the presence of Fe^{3+}-EDDHA) neither in SFCWW showed any change along the treamtent time (data not shown). Solar UVA-irradiance (280–400 nm) was continuously monitored using a pyranometer (Kipp&Zonen, CUV5, Netherlands) which provided data in terms of Wm^{-2}. Inactivation results showed in graphs are the average values of two replicates with standard deviation as error bar against the solar UVA-dose (Wh m^{-2}) received during the solar exposure.

A kinetic analysis was done fitting the experimental data following a log-linear decay according to the Chick´s law Eq. (1) (Model 1) or a 'shoulder phase or lag stage' given by constant bacteria concentration followed by a log-linear decrease, attributed to loss of cells viability after the accumulation of damages Eq. (2) (Model 2). Kinetic data are shown in Table 1 (SI-Supplementary information).

$$Log\left(\frac{N}{N_0}\right) = -k \cdot t \quad (1)$$

$$Log\left(\frac{N}{N_0}\right) = -k \cdot t \begin{cases} 0; \ t \le t_{lag} \\ -k \cdot (t - t_{lag}); \ t > t_{lag} \end{cases} \quad (2)$$

Where N/N_0 represents the bacteria concentration reductions, k is the disinfection kinetic constant (min^{-1}), t is the time of treatment (min), t_{lag} is the duration of the shoulder phase or lag stage (min).

3. Results and discussion

3.1. Photostability analysis of Fe^{3+}-EDDHA

The stability of Fe^{3+}-EDDHA in solution at concentration of 6.2 mg L^{-1} of iron was investigated by following the concentration of dissolved iron ($[Fe]_{ds}$) and the UV–vis absorbance spectrum at 0, 60, 120 and 180 min in the dark and exposure to 30 Wm^{-2} of constant UVA irradiance with and without H_2O_2.

In the dark, the fertilizer solution alone and in the presence of H_2O_2 did not show any significant change on both parameters. Under irradiance, the UV–vis spectrum of the commercial fertilizer solution alone and with H_2O_2 (at Fe/H_2O_2 concentration ratio of 1:2) is shown in Fig. 1. In general, with and without H_2O_2, at time 0 min, the UV–vis spectrum of the iron chelate solution exhibits the typical ligand

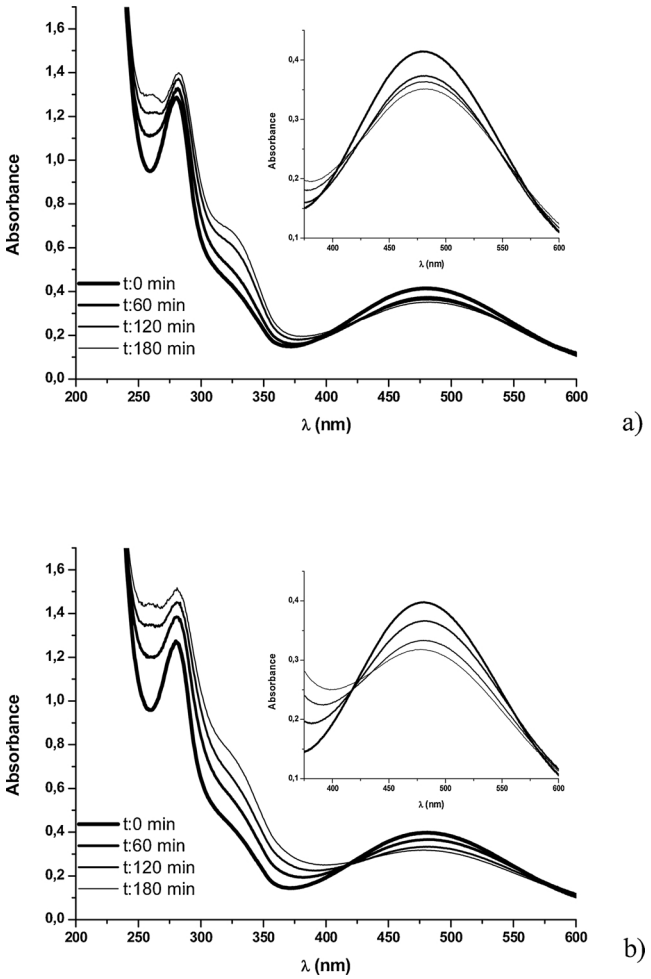

Fig. 1. Absorbance spectrums of a) Fe^{3+}-EDDHA and b) Fe^{3+}-EDDHA/H_2O_2 exposure for 180 min at 30 Whm^{-2} of solar irradiance. Insert graphs shown an extended view of the absorbance spectrum in the range.400–600 nm.

absorptions bands around 200 nm (benzene ring), 281 nm (ortho substitution in the ring) and 482 nm (Fe-phenolate bond) [17]. Throughout irradiance time, the ligand peaks absorbance (200 and 281 nm) increases whereas the peak corresponding to Fe-ligand binding (482 nm) decreases; even in the presence of H_2O_2 this last peak suffers a hypsochromic shift of 4 nm (varying from 482 to 478). These changes are attributed to the decomposition mechanisms (deferration process) of the iron chelate, where the photoexcitation of the complex generates a redox reaction by a single electron transfer from one carboxylate group of the ligand to Fe^{3+}. This leads the reduction of Fe^{3+} to Fe^{2+} and the formation of carboxylate radical cation species that generate photofragmentation products, ending in the formation of Fe^{2+} and partially decomposed organic ligands. In addition, the $[Fe]_{ds}$ measured supports this affirmation as it decreased 15% and 24.8% after 180 min of irradiation for iron chelate solution alone and with H_2O_2. This behaviour agrees with previous studies reporting the photosensitivity of Fe^{3+}-EDDHA [15]. On the other hand, the shift of the Fe-phenolate band can be attributed to the photodegradation of the less stable diastereoisomer (*meso*) present in the fertilizer [18,19].

3.2. Bacterial inactivation by solar disinfection, Fe^{3+}/solar and Fe^{3+}-EDDHA/solar

The inactivation profile and kinetic rates of *E. coli* and *S. enteritidis* by solar photo-inactivation alone and at several iron concentrations of Fe^{3+} from iron salt (Fe^{3+}/solar) and the commercial fertilizer (Fe^{3+}-EDDHA/solar) under natural solar radiation in IW is shown in Fig. 2 and SI-Table 1, respectively. Three iron concentrations were investigated under natural sunlight, 0.5, 2.5 and 5 mg L^{-1}. Dark tests previously performed did not show any effect on the bacterial viability. Best bacterial inactivation rate was obtained with 0.5 mg L^{-1} Fe^{3+}-EDDHA/solar. DL was achieved with a solar UVA-Dose of 25.5 and 21.3 Whm^{-2} (35 and 30 min) for *E. coli* and *S. enteritidis*, respectively, which means a two and four times reduction compared to solar photo-inactivation (*E. coli*: 75 min and 59.3 Whm^{-2}; *S. enteritidis*: 120 min and 96.4 Whm^{-2}).

The inactivation profiles of both bacteria showed a similar trend regarding the following results: i) At same iron concentration tested, the use of chelated iron reached faster inactivation kinetic rates compared with traditional iron salt and ii) The higher the Fe^{3+} concentration added to the sample, the lower the bacterial inactivation kinetic rate, showing a marked limited kinetic rate compared with solar photo-inactivation process. These observations can be explained simultaneously by the amount of dissolved iron concentration ($[Fe]_{ds}$) remained in the water in both processes (SI-Table 2). At the end of the solar process, the $[Fe]_{ds}$ was in all cases < 0.1 mg L^{-1}; meanwhile, for Fe^{3+}-EDDHA/solar process the 50%, 62% and 82% of the initial added iron was kept dissolved for 0.5, 2.5 and 5 mg L^{-1}, respectively. It is widely accepted that the combination of solar radiation with ferric iron can increase the inactivation rate through the formation of exciplexes between Fe^{3+} and some organic compounds of the cell wall, which may contribute to the bacterial inactivation by direct oxidation of the membrane constituents or indirect oxidation by the generation of Fe^{2+}, H_2O_2 and $HO^{\bullet}$ near to the cell wall [20]. Nevertheless, this inactivation enhancement was not observed in the case of Fe^{3+}/solar process for both pathogens. This effect may be explained by the absence of dissolved iron in solution. In addition, although there is some controversy about the activity of iron oxyhydroxides for bacterial disinfection [21], in our experimental conditions and reagent´s concentrations it is possible that the precipitated iron reduced the light penetration and acted as a protective screen for bacteria against solar photons, limiting therefore the bacterial inactivation [20].

Fig. 3 shows the inactivation of *E. coli* and *S. enteritidis* in SFCWW. In this case, solar UVA-dose and treatment time required to achieve the DL was higher for both pathogens compared to inactivation results in IW, which is attributed to the presence of organic matter (25 mg L^{-1} of DOC) and turbidity (100 NTU), parameters that limit or reduce the efficiency in photo-disinfection processes and reinforce the need to investigate the efficiency of these processes under near real conditions. Regarding $[Fe]_{ds}$, a similar behaviour was observed in SFCWW compared to IW (SI-Table 2).

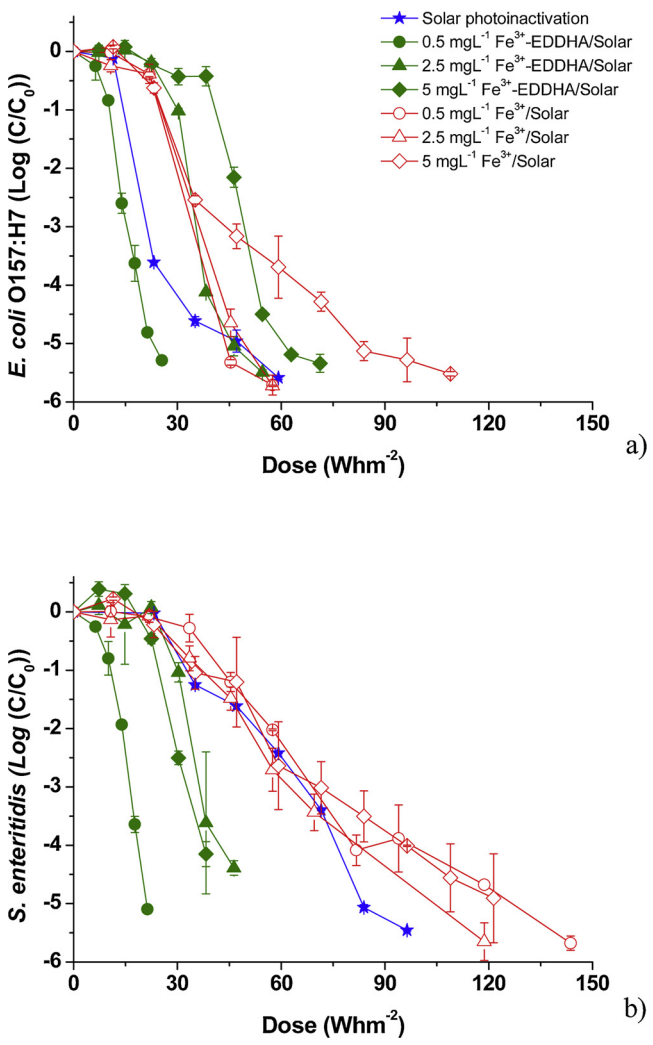

Fig. 2. *E. coli* (a) and *S. enteritidis* (b) inactivation by Fe^{3+}-EDDHA/Solar and Fe^{3+}/solar in isotonic water (IW).

The inactivation kinetic rates of *E. coli* (Fig. 3a), did not show a significant enhancement for all the processes and conditions tested regarding solar photo-inactivation. Nevertheless, *S. enteritidis* results (Fig. 3b) showed that inactivation by Fe^{3+}-EDDHA/solar process was significantly faster (116.4 Whm^{-2}) than Fe^{3+}/solar (137.4 Whm^{-2}) and solar photo-inactivation (169.9 Whm^{-2}), which suggest a different susceptibility between both bacteria against Fe^{3+}-EDDHA. This different response on the inactivation resistance between both bacteria can be explained by structural differences which could play a role in the inactivation mechanism. This aspect will be deeply discussed in the next sections where it is proposed the inactivation mechanisms by Fe^{3+}-EDDHA.

3.3. Bacterial inactivation by Fe^{3+}-EDDHA/H_2O_2/solar, Fe^{3+}/H_2O_2/solar and H_2O_2/solar

The comparative analysis of inactivation profiles and kinetics rates of both bacteria by Fe^{3+}-EDDHA/H_2O_2/solar, Fe^{3+}/H_2O_2/solar and H_2O_2/solar in IW and SFCWW are shown in Figs. 4 and 5 and SI-Table 1.

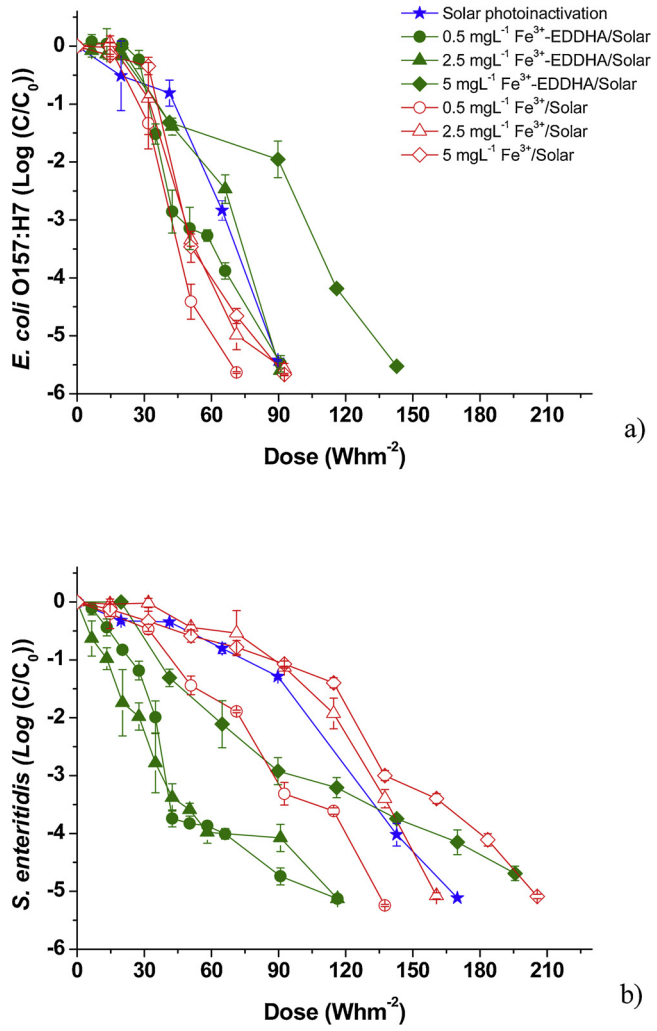

Fig. 3. *E. coli* (a) and *S. enteritidis* (b) inactivation by Fe^{3+}-EDDHA/Solar and Fe^{3+}/solar in synthetic fresh-cut wastewater (SFCWW).

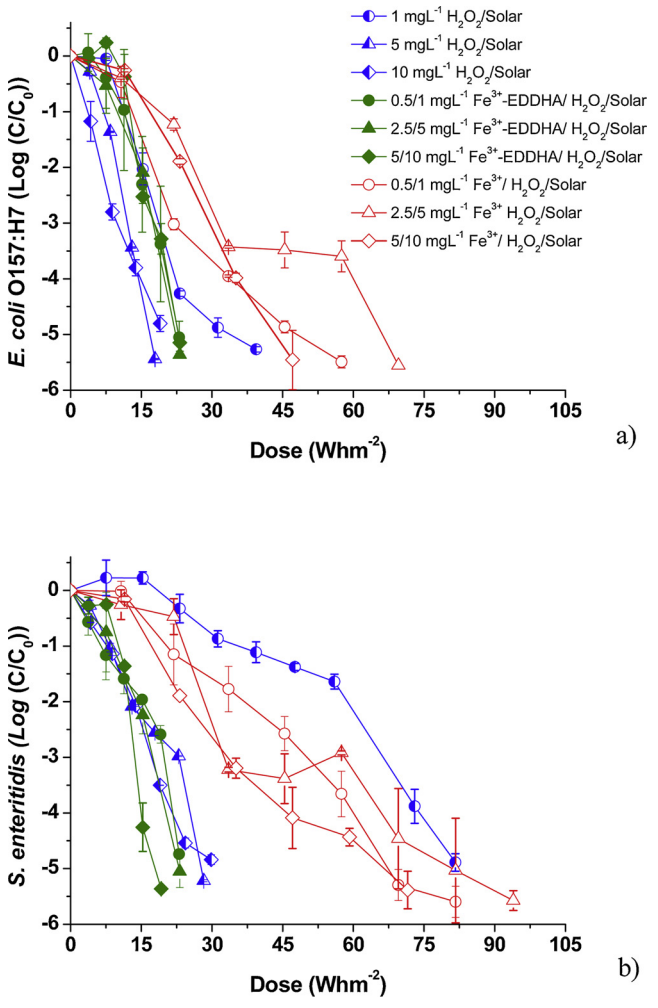

Fig. 4. *E. coli* (a) and *S. enteritidis* (b) inactivation by Fe^{3+}-EDDHA/H_2O_2/solar, Fe^{3+}/H_2O_2/solar and H_2O_2/solar in isotonic water (IW).

In IW (Fig. 4a,b), in the absence of any chemical influence, the trend of inactivation regarding Fe^{3+}/H_2O_2/solar and Fe^{3+}-EDDHA/H_2O_2/solar was similar to the obtained in Fig. 2, but reaching DL with lower solar UVA-dose (or treatment time) and showing a marked decreased of the lag stage attributed to the presence of H_2O_2 (SI-Table 1). In fact, H_2O_2/solar was investigated herein in order to determine the effect of this well-known process on the bacteria viability to discard and/or discuss the overlapping effects on the interpretation mechanisms of bacterial inactivation by Fe^{3+}-EDDHA system. In IW, best inactivation rate was obtained with H_2O_2/solar process with 5 mg L^{-1} of reagent, reaching DL with 17.9 Whm^{-2} (40 min) for *E. coli* and Fe^{3+}-EDDHA/H_2O_2/solar with 5/10 mg L^{-1} of reagents for *S. enteritidis* (19.2 Whm^{-2}, 40 min). Conventional photo-Fenton process showed lower inactivation kinetics compared with Fe^{3+}-EDDHA/H_2O_2/solar and H_2O_2/solar for all concentrations tested in IW. This result coincides with other works reporting bacterial inactivation by solar photo-Fenton at near neutral pH using a low amount of added iron in the solution (< 20 mg L^{-1}) [21,22]. In these cases, the limited inactivation of solar photo-Fenton at neutral pH was attributed to the low amount of iron added, the almost zero $[Fe]_{ds}$ remaining in the sample (SI-Table 2), the lower activity of precipitated iron as oxyhydroxides compared with dissolved iron and the possible reduction of solar photons incoming in the sample by the turbidity generated. All these parameters acting together determine a notable reduction on the capability of solar photo-Fenton for bacterial inactivation. Therefore, if inactivation efficiencies are compared with H_2O_2/solar under similar H_2O_2 concentrations, it has been observed the same or even lower inactivation kinetics for solar photo-Fenton than for H_2O_2/solar [20,22]. This effect can be attributed to the no limitations of H_2O_2 to generated damages on bacteria in the H_2O_2/solar process, as the efficiency of the process depends mainly on the capability of each bacterium to resist the internal damages induced by this solar process [20].

The inactivation results in SFCWW (Fig. 5b) showed that DL (> 5-log reduction) was achieved in all cases but with a slight delay compared with IW, which can be also attributed to the presence of DOC and turbidity. A clear enhancement of bacterial inactivation was obtained from the process Fe^{3+}-EDDHA/H_2O_2, reaching the faster inactivation kinetics rate ($k_{E.coli}$: 0.173 ± 0.011 min^{-1} and $k_{S.enteritidis}$: 0.150 ± 0.033 min^{-1}) with 2.5/5 mg L^{-1} of reagents concentration, requiring 31 Whm^{-2} of solar UVA-dose or 45 min of treatment time.

In both water matrix, again a high $[Fe]_{ds}$ still remained detected in the sample during Fe^{3+}-EDDHA/H_2O_2 process while with Fe^{3+}/H_2O_2, $[Fe]_{ds}$ was lower than 0.1 mg L^{-1}.

In addition, the increase of iron and H_2O_2 concentrations did not show a significant disinfection enhancement neither one nor the other pathogen.

3.4. Interpretation of the bacterial inactivation mechanisms by Fe^{3+}-EDDHA

The inactivation of bacteria by solar photons, H_2O_2/solar and Fe^{3+}/

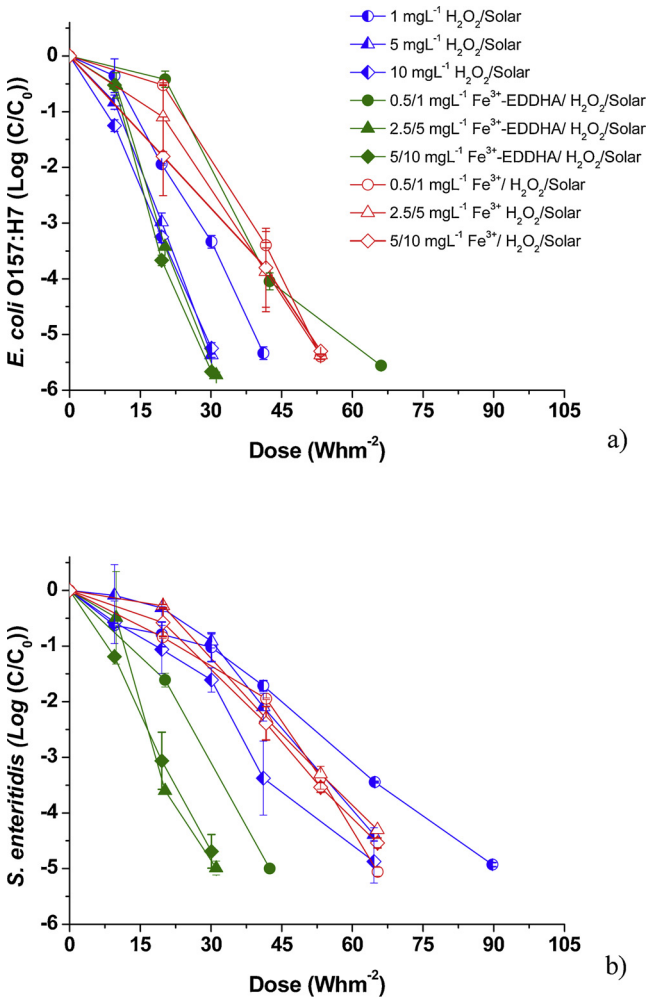

Fig. 5. *E. coli* (a) and *S. enteritidis* (b) inactivation by Fe^{3+}-EDDHA/H_2O_2/solar, Fe^{3+}/H_2O_2/solar and H_2O_2/solar in synthetic fresh-cut wastewater (SFCWW).

solar is very well known and widely explained in literature [20]. Briefly, these mechanisms are based on DNA damage for a combination of direct photo-oxidative damage (solar photo-inactivation) with internal oxidative damage by reactive oxygen species (ROS) generated by internal photo-Fenton reactions between bacterial iron and H_2O_2 from internal presence (metabolic activity and natural occurring iron) or freely diffusing inside of the cell when added to the sample [20].

The proposed mechanisms to explain the enhanced bacterial inactivation by Fe^{3+}-EDDHA/solar process are summarized in Fig. 6.

On one hand (Fig. 6a), it is widely demonstrated that aminopolycarboxylic acid ligands including EDTA provoke changes in the permeability of the outer membrane altering the homeostasis of the cell and eventually end on cell death [23]. Briefly, this change is attributed to the chelation of cations (Ca^{2+} and Mg^{2+}), which purpose is to stabilize electrostatically the different parts of the lipopolysacharides (LPS) present in the surface of the outer membrane, negatively charged by its polyanionic nature. Recently, a functional complexation study reported values of EDDHA affinity for Ca^{2+} and Mg^{2+} higher than other aminopolycarboxylic acid ligands [24]. Moreover, Hernandez-Apaolaza et al reported that salicylaldehide, salicylic acid and salicylaldehydeethylenediaminediimine are Fe^{3+}-EDDHA photodegradation products that can chelate iron [15]. Previous studies reported the ability of acetilylsalicylate to disrupt the outer membrane and changes it permeability [25]. Therefore, in our experimental conditions, although not experimentally determined, it cannot be discard that the free EDDHA or any other subproducts with chelating capacity may

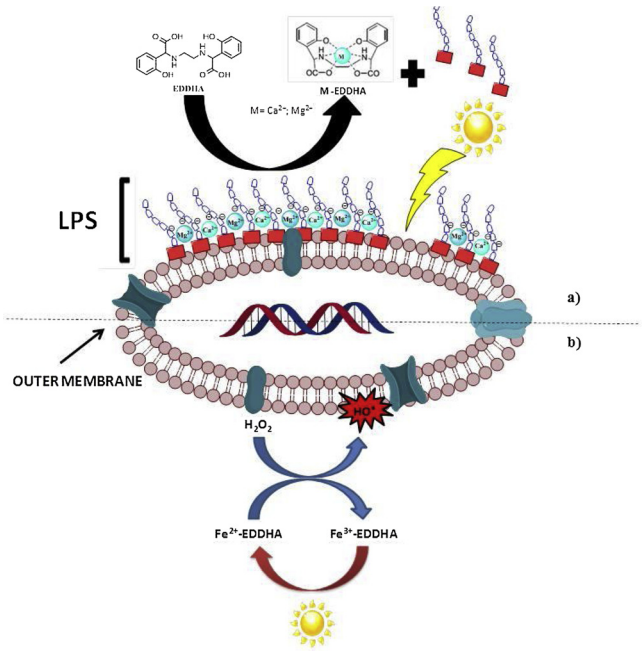

Fig. 6. Proposed inactivation mechanism for Fe^{3+}-EDDHA/solar (a) and Fe^{3+}-EDDHA/H_2O_2/solar processes (b).

affect the membrane permeability making the bacteria more susceptible to be inactivated by solar radiation.

The increase of iron-chelate concentration (from 0.5 to 5 mg L^{-1}), did not show an increase in inactivation rates for neither of the pathogens. This effect can be explained by the screen effect of solar photons (higher at raised concentration of reagent) and also by the limited concentration of Ca^{2+} and Mg^{2+} available in the membrane (10^{-3} - 10^{-4} fg/fg in *E. coli*), and therefore, the increase of chelate concentration will not determine an increased effect on the bacterial susceptibility [15,26]. Moreover, considering the limitation of cations, in SFCWW, the presence of Ca^{2+} and Mg^{2+} can also reduce the efficiency of the Fe^{3+}-EDDHA/solar process through a competition for the chelating agent with the membrane metals [27].

S. enteritidis has been reported to show a higher resistance to be inactivated under stress conditions than *E. coli* [4]. In this work, the inactivation results showed same behaviour except when Fe^{3+}-EDDHA is added to the sample. This curious behaviour could be also explained by the proposed mechanism, considering different membrane stability between both pathogens. Ciesielski et al., reported that the dissociation constant (K_d) of LPS on *E. coli* is an a higher order of magnitude than on *S. enteritidis* [28]. The higher membrane stability of *E. coli* may explain its higher resistance to be affected or inactivated in particular by the presence of Fe^{3+}-EDDHA. Therefore, the different resistance of both bacteria reinforces the currently approach suggested in literature about the need to tests other microorganisms apart from *E. coli* to determine the efficiency of a water disinfection treatment [12].

In the case of Fe^{3+}-EDDHA/H_2O_2/solar there is some controversy about the photochemically or chemically induced electron transfer processes due to low reduction potential (E: -0.560 V) in comparison with Fenton reagents (E: +0.460 V) and Fe^{3+}-EDTA (E= +0.120 V) [29]. Nevertheless, a recent study, reported the use of Fe^{2+}-EDDHA/H_2O_2 as Fenton treatment for degradation of polychlorinated biphenyls (PCBs) in contaminated soils [30], and concluded that the oxidation mechanism of this chelate is based on a catalytic cycle Eqs. (3) and (4). In addition, in our experimental conditions, the presence of light will favors the reduction of Fe^{3+} to Fe^{2+}, closing the cycle Eq. (5) similarly to the mechanism of other iron chelates (EDDS) reported previously and based in the generation of the oxidant species, mainly $HO^{\bullet}$ and also other ROS like $O_2^{\bullet -}$ [20,22].

$$Fe^{2+} - \text{EDDHA} + H_2O_2 \rightarrow Fe^{3+} - \text{EDDHA} + HO^- + HO^{\bullet} \quad (3)$$

$$Fe^{3+} - \text{EDDHA} + H_2O_2 \rightarrow Fe^{2+} - \text{EDDHA} + 2H^+ + O_2^{\bullet -} \quad (4)$$

$$Fe^{3+} - \text{EDDHA} + h\nu \rightarrow Fe^{2+} - EDDHA^{\bullet} \quad (5)$$

In this study, the photo-degradation of the chelate was demonstrated (Fig. 1) but also the generation of $HO^{\bullet}$ in Fe^{3+}-EDDHA/H_2O_2/solar has been confirmed by the detection of phenol using benzene as probe molecule (Fig. S1-1). Summarizing, the main inactivation mechanisms of both bacteria by Fe^{3+}-EDDHA/H_2O_2/solar could be attributed to the accumulative damages on the external-cell membrane by i) the $HO^{\bullet}$ generated during the solar process and ii) the presence of the chelating agent (EDDHA) that changes membrane permeability leading to its degradation and accelerating the bacterial inactivation (Figs. 4 and 5) compare to Fe^{3+}-EDDHA/solar (Figs. 2 and 3).

4. Conclusions

The capability of a commercial iron chelate Fe^{3+}-EDDHA as promoter of bacterial inactivation in wastewater and in combination with natural solar radiation has been demonstrated.

E. coli O157:H7 and *Salmonella sub enteritidis* have been successfully inactivated in IW after 35 min of solar exposure requiring very low concentration of Fe^{3+}-EDDHA (0.5 mg L^{-1}) and reducing the treatment time two and four times compared to solar photoinactivation process.

The presence of organic carbon and high turbidity (100 NTU) in SFCWW delays the bacterial inactivation rate compared with their absence in terms of treatment time and solar UVA dose but reaching DL (> 5-log reduction) in all cases.

Employing commercial fertilizer as Fenton reagent is more efficient than the conventional use of iron salts for the two water matrix studied: IW and SFCWW. The combination of the iron chelate with H_2O_2 (Fe^{3+}-EDDHA/H_2O_2/solar) clearly improves the inactivation efficiency respect to all the treatments tested obtaining very successful inactivation rates (> 5-log reduction). Best bacterial inactivation was obtained in only 45 min using low reagent concentrations (2.5/5 mg L^{-1} of Fe^{3+}-EDDHA/H_2O_2).

The implementation of this process to treat fresh-cut wastewater will allow the reduction of the water footprint thanks to WW reuse for irrigation providing simultaneously the needed iron fertilizer to avoid the iron chlorosis in calcareous soils. In this way, two important agriculture problems in arid and semiarid regions are targeted: water scarcity and iron chlorosis.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.apcatb.2019.04.041.

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